Is p53 an Oncogene or a Tumor Suppressor?

by Jeffrey H. Simonson

Introduction

The gene called *P53* is a major player in cancer. After damage to their DNA, normal cells increase production of p53 protein which acts as a transcription factor increasing production of p21. Activation of Cyclin-CDK is then inhibited by p21. Without Cyclin-CDK activation, the Rb-E2F complex does n dissociate, so E2F cannot activate genes required for transition from G_1 to S phase. Therefore, the cells normally wait while their DNA is repaired before replication. Cells with malfunctioning p53 will continue the cell cycle and replicate mutations. This loss of cell cycle control leads to cancerous tumors.

Original research using DNA-virus-infected tumor cells suggested *P53* was an oncogene (a hyperactive stimulator) able to produce tumors in heterozygous mutants expressing high levels of p53 protein. Later work on aneuploid cells showed *P53* to act as a tumor suppressor (an inactive inhibitor) because all copies of *P53* must be mutant, or missing, for tumor formation. Viruses normally signal host cells to start replication and use the cell's proteins to copy itself. Occasionally, genes from DNA viruses get integrated into the host's genome and a viral gene signaling replication is expressed. Some viral proteins do this by binding to, and inhibiting, cell cycle regulating proteins. This is known to occur when a *papillomavirus* protein inhibits Rb and disrupts cell cycle control. Here I propose to test the hypothesis that p53 is inactivated by an incorporated viral gene product and causes *P53* to act as if it were an oncogene.

Under this viral-inactivation model, DNA-virus-infected tumor cell lines will exhibit a different mechanism for disabling p53 than aneuploid cell lines. Vi infected cells will contain two normal *P53* alleles and a normal amount of p53 protein. They will also have a genome-integrated viral gene whose unknow viral protein (UVP) product binds to p53 protein. In these cells, UVP will bind to p53 and prevent p53 from activating transcription of p21. Conversely, aneuploid tumor cells will have just a single, mutated *P53* gene. The p53 protein will most likely be truncated and in low concentrations. This mutant p53 protein will not be able to activate transcription of p21 either. Neither cell line will arrest in G_1 when DNA damage occurs and *P53* is upregulated. A mutation in only one P53 allele of a viral-infected cell will appear as a dominant-effect oncogene because all p53 proteins are inactivated. In other word even introducing a normal *P53* allele will not rescue the defect; however, this would work in the aneuploid cells.

Methods

I propose to test this viral-inhibition hypothesis using the following molecular tools:**1)** probe host cell genomes for viral genes; **2)** probe host cell genomes for *P53* alleles; and **3)** use antibodies to assess concentrations p53 proteins. The viral gene search will involve probing a Southern blot for copies of viral genes, particularly *UVP*, incorporated into the host's genome. I will also probe genomic Southern blots for presence of *P53* alleles. These DNA probes will consist of a set of labeled oligonucleotides able to detect known *P53* alleles. I will use the p53 antibodies to determine concentrations of p53 protein in each cell type. Since the most common type of non-functional p53 protein is truncated, I will use antibodies for both the N-terminus and the C-terminus p53. I will also use a fluorescent cell sorter to verify mutant phenotypes in both types of cell lines. The sorter is able to isolate cells in either M/G_1 or S/G_2 by detecting the higher brightness of fluorescently-labeled DNA due to replicated chromosomes in S/G_2. Cell lines not exhibiting a majority of cells in M/G_1 phase (particularly after DNA damage) are presumed defective in cell cycle control. If this work continues to a second phase, I will also use a two-hybri system to assay p53 and UVP protein interaction along with transfection techniques to determine which genes can rescue the mutant phenotypes.

Expected Results

Previous studies indicate the host's genome is unlikely to have any homologous genes not introduced by the virus. Therefore, I expect the viral-infected cel lines have viral genes and the aneuploid cells do not. I also expect viral-infected lines to have two normal copies of *P53*; the aneuploid cells should have one copy of a mutant *P53* allele. Viral-infected cell lines are likely to have twice as much p53 protein as aneuploid cells, though this is dependent on how *P5* is regulated. Western blots using amino-terminal antibodies will detect normal p53 in viral-infected cell lines and are likely to detect half as much protein i the aneuploid cells. Since p53 is most commonly truncated, carboxyl-terminal antibodies will detect all protein in viral-infected cell lines and no p53 i aneuploid cells.

Summary

This study will help characterize the roles of *P53* and DNA viruses in cancerous tumor formation. the information gained here may lead to more effective prevention and treatment of human cancers.